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Research Article

Serum Lactate Dehydrogenase Activity and Inflammatory Cell Counts in Nigerian Cervical or Prostate Cancer Patients: Consideration for Immunotherapeutic Potentials

Yinka Gabriel Okunola,[1] Mutiu Alani Jimoh,[2] Ganiyu Olatunbosun Arinola[1]

[1]Department of Immunology, University of Ibadan and University College Hospital, Ibadan, Nigeria
[2]Department of Radiation Oncology, University of Ibadan and University College Hospital, Ibadan, Nigeria

Abstract

Objectives: Elevated lactate dehydrogenase (LDH) enzyme causes insensitivity to anti-Program Death Ligand-1 (PDL-1) antibody immunotherapy, immunosuppression and negatively predicts therapeutic outcomes in various cancer types. Additionally, numerous studies have highlighted strong association of cellular inflammatory markers with tumour development. Despite these findings, there is a scarcity of reports on the connection between serum LDH activity and cells of inflammation in gender-based cancers.

Methods: Thirty (30) prostate cancer patients and 30 cervical cancer patients with corresponding 30 sex- and age-matched controls were recruited. Total- and differential- white blood cells were counted using haematology autoanalyser. Serum LDH levels were measured using ELISA kit.

Results: Median serum LDH activity was significantly increased in combined cancer cases (prostate+cervical cancers) compared with control. Median serum LDH activity was significantly increased prostate cancer patients compared with corresponding controls. Only monocyte counts were significantly higher in cervical cancer patients compared with prostate cancer patients. Significant correlation existed between monocyte counts or neutrophil counts with LDH activities in prostate cancer patients.

Conclusion: This study concluded that serum LDH enzyme could be exploited as targeted immune checkpoint in the therapy of both cancers (cervical and prostate cancer) and that monocyte or neutrophil counts could be used as cellular markers of LDH activity.

Keywords: Gender-based cancers, immune-checkpoints, inflammatory immune cells

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Cancer is a complex group of diseases that can develop at any organs or tissues. It is characterized by uncontrollable growth and spread of abnormal cells to nearby and distant tissues and organs.[1] There are different types of cancers each with its own unique characteristics and clinical presentations. The most common cancers in men are prostate, lung, colorectal, stomach and lung cancer while cervical, breast, colorectal, lung and thyroid cancer are the most common cancers in women. Prostate cancer is the second most common diagnosed cancer in men after lung cancer, and the fifth leading cause of death worldwide.[2] Cervical cancer ranks as the fourth most prevalent

Address for correspondence: Ganiyu Olatunbosun Arinola, Ph.D. Department of Immunology, University of Ibadan and University College Hospital, Ibadan, Nigeria
Phone: +234-80-9812-1520 **E-mail:** drarinolaog64@yahoo.com, drogarinola64@gmail.com

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form of cancer among women globally, and second most common cancers among Nigerian women after breast cancer.[2] The profound burdens and prevalence imposed by prostate and cervical cancers underscore the critical necessity to advance diagnostic mechanisms and treatment strategies.

In cancer, metabolic changes promote glucose usage and lactate production to promote uncontrolled proliferation.[3] This reaction is catalysed by LDH.[4] Elevated serum LDH have been correlated with unfavorable prognosis and negative therapeutic outcome across various cancer types including prostate, cervical, pancreatic and thoracic cancers.[5-8] Also, chronic inflammation is a major risk factor in carcinogenesis[9, 10, 12, 13] and resistance to cancer therapy.[11] Moreover, serum LDH level have been positively associated with inflammatory responses[11, 14] and numerous inflammation based strategies have been suggested as therapeutic target for cancers affecting both males and females.[10, 12, 13, 15] This proposition is reinforced by the recognition of sex-specific variations influencing the incidence, malignancy, and mortality rates of cancers.[1, 10, 13, 15] However, there are limited studies linking serum LDH, cellular inflammation markers with gender-based cancers. Hence, this study seek to fill this gap by evaluating serum LDH activity and inflammation cell counts in patients with cervical or prostate cancer, given the distinct sex dependent pathophysiology and outcomes of these malignancies.

Methods

This is a case-control study. A total of 30 patients with prostate cancer (aged 30 to 65 years) and 30 patients with cervical cancer (aged 25 to 70 years) diagnosed using clinical and laboratory investigations by consultant oncologist in Radiation Oncology Department, University College Hospital, Ibadan, Nigeria, were randomly recruited for the study with 25 corresponding age-matched control for each case. Venous blood sample (5ml) was collected from each study participant, 3ml was dispensed into tube containing anticoagulants and the remaining 2ml was dispensed into plain tube without anticoagulants to obtain serum. The blood samples were gently mixed to ensure adequate homogenization of the anticoagulant. Serum obtained from blood in plain tube was stored at -20°C before used for serum LDH activity determination while blood in bottle containing anticoagulant was used for blood cell counts (total white blood cell, lymphocyte, monocyte, neutrophil, and platelet) using haematology autoanalyser (Sysmex XN-450) immediately after collection.

Serum LDH levels of each sample were measured using LDH ELISA kit (Fortress Diagnostics Limited, United Kingdom). Working reagent was prepared by mixing 5 volumes of R1 Buffer (325 mmol/l of 4-aminomethylpropanol and 63 mmol/l of Lithium lactate) with 1 volume of R2 NAD (50 mmol/l of NAD). A volume (1000µl) of the working reagent and 20µl of the sample were pipetted into a cuvette, mixed and incubated at 37°C for 90 seconds. The absorbance was measured at wavelength of 340nm against distilled water. LDH activity was calculated using absorbance measured after incubation at 90 seconds, absorbance measured after 1 minutes, absorbance measured after 2 minutes, and absorbance measured after 3 minutes.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Science (SPSS) software, version 27.0 tool. Gaussian distribution of the parameters was accessed using histogram with normal curve. Mann-Whitney U and Kruskal Wallis tests were used to determine differences in median values between the groups (in variables with non-Gaussian distribution). Spearman correlation test was used to test for correlations between the parameters. P-values less than 0.05 were considered to be statistically significant.

Ethical Consideration

The study was approved by the University of Ibadan/University College Hospital (U.I./U.C.H.) Joint Ethics Review Committee (UI/EC/0065). Informed consent was obtained from each study participant before enrolment into the study.

Results

In Table 1, LDH activity was significantly higher in either (prostate or cervical) or combined cancer cases (prostate + cervical) compared with all controls. LDH activity was significantly higher in prostate cancer cases compared with prostate control. LDH activity was not significantly different when cervical cancer cases were compared with cervical cancer controls or when prostate cancer cases compared with cervical cancer cases. LDH activity was significantly higher in cervical cancer control (apparently healthy normal females) compared with prostate control (apparently healthy normal males). Total white blood cell, neutrophil, monocyte and lymphocyte counts were lower in prostate cancer patients compared with cervical cancer patients but only monocyte counts showed significant difference (Table 2). Neutrophil counts showed significant negative correlation while monocyte counts showed significant positive correlation with LDH activities in prostate cancer patients. None of the white blood cell counts showed significant correlation with LDH activities in cervical cancer patients (Table 3).

Table 1. LDH activities in prostate cancer patients, cervical cancer patients and the control

LDH activity (U/L)	Groups	p
All Cases (n=60)	All Controls (n=50)	
234.76 (202.38 – 288.72)	205.07(171.34–1.26)	<0.001*
Prostate Cancers (n=30)	All Controls (n=50)	
244.20 (204.40 – 269.83)	205.07(171.34–221.26)	<0.001*
Cervical Cancers (n=30)	All Controls (n=50)	
226.66 (184.84 – 305.59)	205.07(171.34–221.26)	<0.001*
Prostate Cancer (n=30)	Prostate Control (n=25)	
244.20 (204.40 – 269.83)	167.30 (180.79–207.10)	<0.01*
Cervical Cancer (n=30)	Cervical Control (n=25)	
226.66 (184.84 – 305.59)	218.57 (199.68 –233.41)	0.208
Prostate Cancer (n=30)	Cervical Cancer (n=30)	
244.20 (204.40 – 269.83)	226.66 (184.84– 305.59)	0.959
Prostate Control (n=25)	Cervical Control (n=25)	
167.30 (180.79 – 207.10)	218.57 (199.68–233.41)	0.007*

LDH: Lactate Dehydrogenase. *Statistically significant different. Values are presented as median (interquartile range).

Table 2. Blood cell counts in prostate cancer patients compared with cervical cancer patients

Parameters (×10^9/L)	Prostate cancer (n=30)	Cervical cancer (n=30)	p
Total WBC	4.95 (4.30 – 11.40)	5.90 (4.78 – 6.78)	0.722
Neutrophils	2.40 (2.10 – 4.78)	3.50 (2.65 – 6.78)	0.819
Lymphocytes	2.40 (1.90 – 6.45)	3.25 (1.60 – 6.73)	0.610
Monocytes	0.10 (0.05 – 0.20)	1.00 (0.50 – 2.00)	0.011*
Platelets	212.50 (170 – 326)	205.00 (153– 283)	0.988

Values are presented as median (interquartile range). *Statistically significant different.

Table 3. Correlation of serum LDH activity with blood cell counts in prostate and cervical cancer patients

	Total WBC	Neutrophils	Lymphocytes	Monocytes	Platelets
Prostate cancer patients					
r	-0.195	-0.524	-0.332	0.400	-0.067
p	0.301	0.003*	0.073	0.028*	0.725
	Total WBC	**Neutrophils**	**Lymphocytes**	**Monocytes**	**Platelets**
Cervical cancer patients					
r	-0.175	0.130	-0.192	-0.016	0.295
p	0.356	0.493	0.309	0.933	0.113

*Statistically significant different.

Discussion

Dysregulated glucose metabolism and lactic acid production have been identified as contributors to cancer proliferation and metastasis.[3, 16] Lactate dehydrogenase (LDH) catalyses the conversion of lactate from pyruvate, thus underscoring its negative prognostic potential for cancer development.[4, 5] In the present study, serum LDH was significantly elevated in all cancer cases (prostate and cervical cancers combined) when compared with control group (healthy males and females combined). Furthermore, a significant increase of LDH activities was also observed in prostate cancer patients compared with the corresponding control. This observation aligns with previous

findings of elevated serum LDH levels in various cancer cases.[5-8, 17, 18] Elevated LDH levels are the product of enhanced tumour glycolytic activity, tumor necrosis and hypoxic tumour microenvironment.[4, 19] Treatment with LDH inhibitors (quercetin, diclofenac and lonidamine) were suggested immunonotherapeutic attempts for tumours.[20-22] Another study proposed the use of LDH inhibitor against excessively produced LDH in COVID-19 patients.[23] Moreover, in the treatment of cancers, many natural compounds either solely or in combination with conventional chemotherapeutic drugs have been developed to inhibit LDH[24] or increasing sensitivity to conventional chemotherapeutic drugs.[25] These previous reports[20-25] and present data call for further search for LDH inhibitors as promising therapeutic agents to retard cancer progression. This aforementioned recommendation is based on earlier reports[26, 27] that pyruvate produced during glycolysis is converted into lactate by LDH. The produced lactate is either metabolised into pyruvate in the liver through the Cori cycle[26] or contributes to polarisation of tumor-associated macrophages (TAMs) as M2-phenotype, which favours tumour growth and diminishes anti-tumour functions of phagocytes and T-regs.[27] Furthermore, in situ LDH creates acidic microenvironment through lactate production promoting ion trap which reduces efficacy and bioavailability of certain weak basic chemotherapeutic drugs within tumour cells.[26, 27] The implication of present study is that raised LDH activities in cancer patients might be one of the factors responsible for immune suppression in cancer patients.

To further investigate if LDH targeted immunotherapy will be useful against both male and female cancers; we compared LDH activities in prostate cancer patients with cervical cancer patients. Our study revealed similar LDH activity in patients diagnosed with prostate cancer in comparison to those with cervical cancer. The mechanism responsible for this observation was hypothesised to be similarity of tissue destruction and indifferent inflammation or hypoxia state in the tumour microenvironment of male and female cancers. Also, it might be an indication that cancers in different genders have similar enzyme based mechanism and thus may need similar immunotherapeutic management. In contrast to the findings of similar LDH activity in prostate cancer patients compared with cervical cancer patients, increased level of LDH activity was noted in apparently healthy females compared with apparently healthy males, thus supporting the conjecture that sex hormones might influence LDH activity differently during carcinogenesis compared with normal condition. Though the study on the influence of sex hormones on LDH activity is scarce, but oestrogen was found to increase LDH activity[28] while castration was reported to reduce the total LDH activity and the administration of testosterone propionate restored LDH activity.[29] The observed dissimilarities in LDH activities as observed in our study between males and females during normal state and cancerous condition is likely to go beyond hormonal variations, but could be attributed to genetic factors, enzyme expression profiles and sub-types, or overall metabolic rates.[30] Moreover, the nature of the cancers themselves, such as their stage, aggressiveness and specific molecular characteristics could contribute to variations in LDH activity.[31, 32]

Blood cell based inflammation parameters gained prominence as accessible and minimally invasive indicators of cancer progression and management.[10] Additionally, LDH correlations with markers of systemic inflammation,[6, 14] pointing towards an intricate association between LDH level and cellular inflammatory markers. To further investigate potential gender-specific variations among cervical and prostate cancer patients, we counted total- and differential- white blood cells of cervical and prostate cancer patients. Though, the counts of total white blood cells, neutrophils, lymphocytes, monocytes and platelets were higher in cervical cancer patients than in prostate cancer patients, but only monocyte counts showed statistical significance. These observations align with a report.[10] Our result suggests that sex hormones might contribute to the cellular inflammatory profiles seen in these two types of cancer, with lowering effect in prostate cancer patients compared to cervical cancer patients. When serum LDH activity, white blood cell counts were correlated in both prostate and cervical cancer patients, the results indicated distinct patterns between the two cancers. In cervical cancer patients, none of the blood cell counts showed correlation with serum LDH activity. While in prostate cancer patients, monocyte counts showed significant positive relationship and neutrophil counts showed significant negative correlation with serum LDH activity, thus supporting our earlier suggestion of lower inflammation in prostate cancer compared with cervical cancer patients. Fischer et al.[33] showed that lactic acid derived from tumours and elevated LDH impairs the function of cytotoxic T cells, reduces the production of pro-inflammatory cytokines, and hinders the activity of immune cells, thereby creating an immunosuppressive environment in a concentration dependent manner in our cancer patients. This immunosuppression might be higher in prostate cancer patients than cervical cancer patients as a result of fairly higher LDH activities and relatively lower counts of certain immune cells.

Conclusion

High LDH activities in prostate and cervical cancer patients compared with healthy participants underscore the exploration of LDH inhibitors as potential therapeutic immune checkpoint for cancer patients and that certain inflammatory cells (monocytes and neutrophils) could be considered as tracers of LDH activities.

Limitations of the Study

The study is constrained by a relatively small sample size, which may impact the generalisability of the findings to a broader population. Future study should quantify specific LDH isoenzymes to offer a detailed understanding of the source of LDH elevation in each cancer case since LDH isoenzyme is organ specific.

Disclosures

Ethics Committee Approval: This study was approved by the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee (UI/EC/0065).

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